EUROPEAN PATENT OFFICE

In re Application of:	Medinnov, Inc. et al
International Application No.:	PCT/CA03/01429
International Filing Date:	September 19, 2003
Title:	AN ANALYSER FOR THE SIMULTANEOUS ENZYMATIC DETECTION OF CLOSELY RELATED ANALYTES
Docket:	EKS/13310.4

September 7, 2004

AMENDMENT AND RESPONSE TO THE WRITTEN OPINION

EUROPEAN PATENT OFFICE
c/o International Preliminary Examining Authority
P.B. 5818 Patentlaan 2
NL-2280 HV Rijswijk – Pays Bas

Attn: Jenkins, G.

Dear Madam, Dear Sir,

In reply to the Written Opinion of June 9, 2004, kindly amend the application as follows.

IN THE CLAIMS

Kindly substitute the enclosed set of claims for the corresponding set of claims presently on file.

REMARKS

Claims 1-45 remain in the case.

Claim 23 has been amended by suppressing the expression "at least".

Re Item V

2. NOVELTY

In the written opinion of June 9, 2004, the Examiner considered that claims 1-3, 9, 10, 23-25, 27-29, 35 and 36 lack novelty in view of D1 and D3, pursuant to Art. 33(2) PCT.

Applicant respectfully traverses the Examiner's objection as follows:

D3: US 5312590 A 1994.05.17 998, vol. 17, no. 6-7, pages 1111 to 1128

D3 teaches a device for multi-species determination from a single sample drop, comprising a support base, a mixed electrode system, an enzymatic reaction means, a detector and a data processor. However, the multi-species determination in this patent is only achieved by using a mixture of several enzymes (cholesterol oxidase, cholesterol esterase and horseradish peroxidase to detect total cholesterol)(column 7, lines 18-21).

In contrast to the teachings of D3, claim 1 (apparatus) and claim 27 (enzymatic reaction monitoring component) of the present application are directed to the simultaneous detection and measurement of the concentration of two related analytes, being substrates for a common enzyme. Therefore, in the present invention, only one enzyme at a time is used for detecting two closely related substrates of that specific enzyme. It is respectfully submitted that this particular limiting characteristic confers novelty to claims 1 and 27, and to their dependent claims 2-3, 9, 10, 28, 29, 35 and 36 in view of D3.

D1: PLEGGE ET AL: 'Analysis of ternary mixtures with a single dynamic microbial sensor and chemometrics using a nonlinear multivariate calibration', ANAL CHEM, 01. June 2000, vol. 72, no. 13, pages 2937 to 2942.

Plegge et al. teach a method for simultaneously measuring the concentration of acetate, L-lactate and succinate comprising: a) reacting a plurality of reference samples having known concentrations and proportions of the analytes, b) establishing a kinetic profile having at least two points for each of the reference samples, and c) reacting a test sample and determining concentrations of the components. However, the reacting steps in this document are performed by using a unique microorganism of the strain *Alcaligenes eutrophus*, which most certainly contains a plurality of different enzymes.

Claim 23 has been amended in limiting the method to exactly two related analytes, being substrates for a common enzyme. It is respectfully submitted that this amendment and

the limiting feature of using only one enzyme confers novelty to claim 23 and its dependent claims 24-25, in view of D1.

3. INVENTIVE STEP

In the written opinion of June 9, 2004, the Examiner considered that claims 4-8, 11-22, 26, 30-34, 37-45 lack an inventive step, pursuant to Art. 33(3) PCT.

Applicant submits the following arguments in traverse of the Examiner's objection. For the purpose of consistency with respect to the above "Novelty" arguments, and for the purpose of completeness, Applicant addresses the Examiner's "Inventive Step" remarks by separately commenting on each independent claim (*i.e.* claims 1, 23 and 27).

Firstly, it is respectfully submitted that claim 1 is inventive in view of the cited prior art, under Article 33(3) PCT.

Regarding claim 1, D3 is considered to be the closest prior art. D3 discloses a device for multi-species determination in a single sample drop, comprising a support base, a mixed electrode system, an enzymatic reaction means comprising a mixture of several enzymes, a detector and a data processor. The multi-species determination tought by D3 is achieved by using a mixture of several enzymes (cholesterol oxidase, cholesterol esterase and horseradish peroxidase to detect total cholesterol).

The additional novelty imparting feature of claim 1 over D3, is that the analyser is specifically designed to simultaneously detect and measure the concentration of two related analytes, being substrates for a common enzyme.

The technical effect of this modification is that only one enzyme is needed to determine the concentration of two analytes.

The problem to be solved by the present invention may therefore be regarded as the provision of a simpler analyser for multi-species determination.

The solution to this problem is to very much restrict the multi-species determination to two species only, which are both substrates for one single enzyme.

D1 describes a method for simultaneously measuring the concentration of three components, namely acetate, L-lactate and succinate, which uses a unique microorganism of the strain *Alcaligenes eutrophus*, and not a single enzyme. Therefore,

the combination of D3 and D1 would not have obviously led a person of skill in the art to the simpler solution as proposed in claim 1.

Similarly, D2 (LIDEN ET AL: 'Rapid alcohol determination in plasma and urine by column liquid chromatography with biosensor detection', J PHARM AND BIOMED ANAL, 01. September 1) describes an enzyme electrode for detection of ethanol and methanol in biological fluids following separation by liquid chromatography. The enzyme electrode in D2 is prepared with at least two different enzymes, namely alcohol oxidase or alcohol dehydrogenase (AOC) and horseradish peroxidase (HRP). Therefore, the combination of D3 and D2 would not have obviously led a person of skill in the art to the simpler solution proposed in claim 1.

In addition, the solution proposed in claim 1 is economically counter intuitive since it allows less determinations at a time.

In light of the foregoing, it is respectfully submitted that claim 1 does involve an inventive step over D3, alone or in combination with D1 or D2. It is further respectfully submitted that dependent claims 2-22 also involve an inventive step over the same prior art documents.

Secondly, it is respectfully submitted that claim 23 is inventive in view of the cited prior art, under Article 33(3) PCT.

Regarding claim 23, D2 is considered the closest prior art. D2 describes an enzyme electrode for detection of ethanol and methanol in biological fluids following separation by liquid chromatography. The enzyme electrode in D2 is prepared using at least two different enzymes, namely alcohol oxidase or alcohol dehydrogenase (AOC) and horseradish peroxidase (HRP).

The additional features of claim 23 over D2 are that the method of the present invention uses kinetic models to obtain the concentration of two related analytes simultaneously from the electrical signal obtained from the biological fluid, with a common enzyme, for which both analytes are substrates.

The technical effects of these modifications are that no time-consuming liquid chromatography step is required to separate the two analytes, and that only one enzyme is needed to determine their respective concentrations.

The problem to be solved by the present invention may therefore be regarded as the provision of a faster and simpler assay for measuring the concentration of two analytes in biological fluids.

The solution to this problem is to choose related analytes, in the sense that they are substrates for a common enzyme, and to use kinetic models to obtain the concentration of both analytes simultaneously.

D1 describes a method for simultaneously measuring the concentration of three components, namely acetate, L-lactate and succinate, which uses a unique microorganism of the strain *Alcaligenes eutrophus*, and not a single enzyme. Therefore, the combination of D2 and D1 would not have obviously led a person of skill in the art to the simpler and faster solution proposed in claim 23.

D3 describes a device for multi-species determination from a single sample drop, comprising a support base, a mixed electrode system and an enzymatic reaction means. The multi-species determination in this patent is only achieved by using a mixture of several enzymes (cholesterol oxidase, cholesterol esterase and horseradish peroxidase to detect total cholesterol). Therefore, the combination of D2 and D3 would not have obviously led a person of skill in the art to the simpler and faster solution proposed in claim 23.

In light of the foregoing, it is respectfully submitted that amended claim 23 does involve an inventive step over D2, alone or in combination with D1 or D3. It is further respectfully submitted that dependent claims 24-26 also involve an inventive step over the same prior art documents.

Thirdly, it is respectfully submitted that claim 27 is inventive in view of the cited prior art, under Article 33(3) PCT.

Regarding claim 27, D3 is considered the closest prior art. D3 describes a device for multi-species determination from a single sample drop, comprising a support base, a mixed electrode system, an enzymatic reaction means, a detector and a data processor. The multi-species determination in this patent is only achieved by using a mixture of several enzymes (cholesterol oxidase, cholesterol esterase and horseradish peroxidase to detect total cholesterol).

The additional feature of claim 27 over D3 is that the monitoring component is specifically designed to simultaneously detect and measure the concentration of two related analytes, being substrates for a common enzyme.

The technical effect of this modification is that only one enzyme is needed to determine the concentration of two analytes.

Very similarly to claim 1, it is respectfully submitted that claim 27 does involve an inventive step over D3, alone or in combination with D1 or D2. It is further respectfully submitted that dependent claims 28-45 involve an inventive step over the same prior art.

Consideration of these remarks is respectfully requested, and issuance of a favourable International Preliminary Examination Report is earnestly solicited.

Respectfully submitted,

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CLAIMS:

1. An analyzer for simultaneously detecting and measuring the concentration of two related analytes, said analytes being substrates for a common enzyme, comprising:

(a) an enzymatic reaction monitoring component including a support base, a mixed electrode system consisting of a working electrode, an auxiliary electrode and a reference electrode, said mixed electrode system being supported by said support base, and an enzymatic reaction means incorporating said enzyme, said enzymatic reaction means being disposed on said mixed electrode system; whereby, when said enzymatic reaction means is placed in contact with a liquid sample containing said two related analytes, said two related analytes chemically react with said enzyme to produce an electronic signal directly related to the concentration of each of said two related analytes in said liquid sample;

(b) a detector including a sensor, said detector being connected to said enzymatic reaction monitoring component and capable of continuously detecting and amplifying said electronic signal to produce amplified signals; and

(c) a data processor capable of converting the amplified signals into numerical data representative of the concentration of each of said two related analytes.

2. An analyzer as defined in claim 1, wherein said working electrode and said auxiliary electrode are composed of platinum, and wherein said reference electrode is composed of silver.

3. An analyzer as defined in claim 2, wherein said enzymatic reaction means comprises a layer of a permeable polymer on which is bound a layer including said enzyme, said layer being deposited on said mixed electrode system, and a protective membrane impregnable with a buffer solution and reagents capable of promoting said enzymatic reaction, said protective membrane being disposed over said layer of a permeable polymer.

4. An analyzer as defined in claim 3, wherein said permeable polymer is selected from the group consisting of polylysine, poly(4-styrene sulfonate), polyethylene glycol, perfluorosulfonic acid polymers and agarose.

5. An analyzer as defined in claim 4, wherein said reagents include electron transfer reagents selected from the group consisting of p-phenylenediamine, peroxidase and ferrocene derivatives.

6. An analyzer as defined in claim 5, wherein said ferrocene derivatives include ferrocene dicarboxylic acid, and ferrocene monocarboxylic acid.

7. An analyzer as defined in claim 6, wherein said buffer solution is selected from the group consisting of phosphates, saline phosphate buffers (phosphates + NaCl), TRIS-HCl, Hepes, with or without EDTA, and a wetting agent such as SDS, Triton X-100 and Tween 20.

8. An analyser as defined in claim 7, wherein said enzymatic reaction monitoring component is a disposable electrode.

9. An analyzer as defined in claim 2, wherein said enzymatic reaction means comprises a reagent well capable of receiving a buffer solution including said enzyme, said liquid sample, and optionally reagents capable of promoting said enzymatic reaction.

10. An analyzer as defined in claim 9, wherein said reagents include electron transfer reagents selected from the group consisting of p-phenylenediamine, peroxidase and ferrocene derivatives.

11. An analyzer as defined in claim 10, wherein said ferrocene derivatives include ferrocene dicarboxylic acid, and ferrocene monocarboxylic acid.

12. An analyzer as defined in claim 11, wherein said buffer solution is selected from the group consisting of phosphates, saline phosphate buffers (phosphates + NaCl), TRIS-HCl, Hepes, with or without EDTA, and a wetting agent such as SDS, Triton X-100 and Tween 20.

13. An analyser as defined in claim 12, wherein said enzymatic reaction monitoring component is a permanent electrode.

14. An analyzer as defined in claims 8 and 13, wherein said enzyme is an oxidase.

15. An analyser as defined in claim 14, wherein said oxidase is alcohol oxidase.

16. An analyzer as defined in claim 15, wherein said related analytes are methanol and ethanol.

17. An analyzer as defined in claim 16, wherein said liquid sample is a biological specimen selected from the group consisting of saliva, blood or serum.

18. An analyzer as defined in claim 17, wherein said support base is composed of any suitable material capable of supporting said mixed electrode system.

19. An analyzer as defined in claim 18, wherein said support base is composed of plastic.

20. An analyzer as defined in claim 8, wherein said analyzer is a portable analyzer.

21. An analyzer as defined in claim 13, wherein said analyzer is a non-portable analyzer.

22. An analyzer as defined in claims 20 and 21, for use in point-of-care units, in laboratories, in police services, in forensic applications and in industrial applications.

23. A method for simultaneously detecting and measuring the concentration of ~~at least~~ two related analytes in a sample, said related analytes being substrates for a common enzyme, wherein said enzyme reacts with said related analytes following specific different reaction kinetics, and wherein said method comprises:

(a) reacting a plurality of reference samples having known concentrations and proportions of said related analytes, said proportions ranging from 0 to 100% of a first analyte to 100% to 0% of another related analyte, with said enzyme;

(b) establishing a kinetic profile having at least two points for each of said plurality of reference samples; and

(c) reacting a test sample comprising an unknown concentration and proportion of said related analytes with said enzyme and determining the concentration of said related compounds in said test sample using said established kinetic profiles.

24. A method as defined in claim 23, wherein said unknown concentration of said related analytes is established using multiple regression analysis of said kinetic profile.

25. A method as defined in claim 23, wherein said unknown concentration of said related analytes is established using reaction kinetics equations.

26. A method as defined in claim 24 and 25, wherein said related analytes are methanol and ethanol.

27. An enzymatic reaction monitoring component for simultaneously detecting and measuring the concentration of two related analytes, said analytes being substrates for a common enzyme, comprising:

(a) a support base;

(b) a mixed electrode system consisting of a working electrode, an auxiliary electrode and a reference electrode, said mixed electrode system being supported by said support base; and

(c) an enzymatic reaction means incorporating said enzyme, said enzymatic reaction means being disposed on said mixed electrode system; whereby, when said enzymatic reaction means is placed in contact with a liquid sample containing said two related analytes, said two related analytes chemically react with said enzyme to produce an electronic signal directly related to the concentration of each of said two related analytes in said liquid sample.

28. An enzymatic reaction monitoring component as defined in claim 27, wherein said working electrode and said auxiliary electrode are composed of platinum, and wherein said reference electrode is composed of silver.

29. An enzymatic reaction monitoring component as defined in claim 28, wherein said enzymatic reaction means comprises a layer of a permeable polymer on which is bound an enzyme layer, said enzyme layer being deposited on said mixed electrode system, and a protective membrane impregnable with a buffer solution and reagents capable of promoting said enzymatic reaction, said protective membrane being disposed over said layer of a permeable polymer.

30. An enzymatic reaction monitoring component as defined in claim 29, wherein said permeable polymer is selected from the group consisting of

polylysine, poly(4-styrene sulfonate), polyethylene glycol, perfluorosulfonic acid polymers and agarose.

31. An enzymatic reaction monitoring component as defined in claim 30, wherein said reagents include electron transfer reagents selected from the group consisting of p-phenylenediamine, peroxidase and ferrocene derivatives.

32. An enzymatic reaction monitoring component as defined in claim 31, wherein said ferrocene derivatives include ferrocene dicarboxylic acid, and ferrocene monocarboxylic acid.

33. An enzymatic reaction monitoring component as defined in claim 32, wherein said buffer solution is selected from the group consisting of phosphates, saline phosphate buffers (phosphates + NaCl), TRIS-HCl, Hepes, with or without EDTA, and a wetting agent such as SDS, Triton X-100 and Tween 20.

34. An enzymatic reaction monitoring component as defined in claim 33, wherein said enzymatic reaction monitoring component is a disposable electrode.

35. An enzymatic reaction monitoring component as defined in claim 28, wherein enzymatic reaction means comprises a reagent well capable of receiving a buffer solution including said enzyme, said liquid sample, and optionally reagents capable of promoting said enzymatic reaction.

36. An enzymatic reaction monitoring component as defined in claim 35, wherein said reagents include electron transfer reagents selected from the group consisting of p-phenylenediamine, peroxidase and ferrocene derivatives.

37. An enzymatic reaction monitoring component as defined in claim 36, wherein said ferrocene derivatives include ferrocene dicarboxylic acid, and ferrocene monocarboxylic acid.

38. An enzymatic reaction monitoring component as defined in claim 37, wherein said buffer solution is selected from the group consisting of phosphates, saline phosphate buffers (phosphates + NaCl), TRIS-HCl, Hepes, with or without EDTA, and a wetting agent such as SDS, Triton X-100 and Tween 20.

39. An enzymatic reaction monitoring component as defined in claim 38, wherein said enzymatic reaction monitoring component is a permanent electrode.

40. An enzymatic reaction monitoring component as defined in claims 34 and 39, wherein said enzyme is an oxidase.

41. An enzymatic reaction monitoring component as defined in claim 40, wherein said oxidase is alcohol oxidase.

42. An enzymatic reaction monitoring component as defined in claim 41, wherein said related analytes are methanol and ethanol.

43. An enzymatic reaction monitoring component as defined in claim 42, wherein said liquid sample is a biological specimen selected from the group consisting of saliva, blood or serum.

44. An enzymatic reaction monitoring component as defined in claim 43, wherein said support base is composed of any suitable material capable of supporting said mixed electrode system.

45. An enzymatic reaction monitoring component as defined in claim 44, wherein said support base is composed of plastic.